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Ionic channel activity induced by fusion of *Rhodospirillum rubrum* chromatophores with a planar bilayer lipid membrane

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Abstract

The present work concerns mechanisms of ionic conductivity of photosynthetic membranes. It is shown that reconstitution of vesicles of photosynthetic membranes (chromatophores) of purple bacteria *Rhodospirillum rubrum* into a planar bilayer lipid membrane leads to fluctuations of current showing the existence of a channel with a predominant conductance of ~ 230 pS in the presence of 100 mM KCl. Measurements under the conditions of KCl gradient prove that this channel is cation selective (P_K/P_{Cl} = 7.2). Voltage inactivation of the channel is demonstrated which is prevented by treatment with trypsin.

Key words: Ionic channel; Chromatophore membrane; Planar lipid bilayer; *Rhodospirillum rubrum*

1. Introduction

It is known that in the photosynthetic membranes of higher plant chloroplasts and phototrophic bacteria, the light energy is converted into the transmembrane electrochemical potential gradient of protons [1]. The nature of ionic fluxes across the membrane which cause relaxation of the membrane potential thereby providing establishment of a steady-state electrical potential difference on the photosynthetic membrane is unclear. This work deals with studying the ionic conductivity of the photosynthetic membrane of purple bacteria.

Ionic channels represent widespread membrane structures facilitating ion movement across a hydrophobic barrier of biological membranes [2,3]. Recently a series of data appeared in favour of the existence of ionic channels in energy-transducing membranes, namely in an inner membrane of mitochondria [4–9], thylakoid membrane of higher plant chloroplasts [10–12], plasma membrane of yeast [13] and cytoplasmic membrane of bacteria [14–17]. Here we present the data on ionic channel recording in chromatophore membranes of purple photosynthetic bacteria fused with planar lipid bilayers.

2. Materials and methods

Chromatophores were isolated from *Rhodospirillum rubrum* cells by sonication, differential centrifugation and subsequent purification by means of centrifugation in a discontinuous sucrose gradient (31–42%) [18].

Bilayer lipid membranes (BLMs) were formed by a conventional technique [19] on a 0.3-mm diameter hole in Teflon partition separating two aqueous compartments of a cell, from a decane solution of 2% phosphatidylcholine (Sigma) and 1% cholesterol (Serva). The chromatophore vesicles were incorporated into BLM in the presence of an osmotic gradient [20] by addition of chromatophores and urea (180–270 mM) to the *cis*-side. It is generally accepted that vesicles fuse opening so that the internal side of the vesicle bilayer (the periplasmic side of the chromatophore membrane in our case) is exposed to the *trans*-side [21–22]. Absorbance of chromatophores in the cell at 880 nm was about 1. The bathing aqueous solution contained 10 mM citrate (pH 6.5), 2 mM $MgCl_2$, 100 mM KCl (unless otherwise stated). Experiments were performed at room temperature.

The electric current recording was performed under voltage-clamp conditions. The sign of the applied potential was such that the *trans*-side was virtual ground. Currents were amplified by a patch-clamp amplifier (Opus, Moscow) with a cutoff frequency of 500 Hz and stored on a videotape after digitizing by Adarec (Opus, Moscow). Computer analysis was carried out using a single-channel analysis program, PAT, supplied by J. Dempster (Department of Physiology and Pharmacology, University of Strathclyde, Glasgow, Scotland, UK) as well as using a similar program of P. Ivanov (Department of Physics, Moscow University).

3. Results and discussion

Fig. 1 illustrates a typical trace of fluctuations of current across BLM after incorporation of *R. rubrum* chromatophores at −100 mV. A single channel with a substrate is observed.

Fig. 2 shows an example of analysis of channels in a typical experiment. It comprises the traces of current across BLM at different values of the applied voltage and current–voltage characteristics of the current transitions calculated from the current–amplitude histograms, which are also presented in Fig. 2. The single-channel conductance determined from the current–voltage de-

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Fig. 1. A trace of current across BLM after fusion with *R. rubrum* chromatophores observed under symmetrical conditions (100 mM KCl at both sides) at the voltage applied of −100 mV.

pendence amounts to 230 ± 11 pS. The experiment was performed firstly under symmetrical conditions (100 mM KCl at both sides). The subsequent determination of the current–voltage dependence in the presence of KCl gradient (280 mM KCl *trans* vs. 100 mM KCl *cis*) allowed us to show that the ionic channels observed possess the cation selectivity. The permeability ratio calculated from the zero current potential according to Goldman-Hodgkin-Katz equation for K^+ and Cl^- (P_K/P_{Cl}) is equal to 7.2 ± 0.9. The discrimination between potassium and sodium ions seemed to be negligibly small, because addition of NaCl to the *cis* compartment making cation concentrations identical at both sides shifted the current–voltage dependence practically to the initial position observed under the conditions of symmetrical KCl.

The pH shift from 6.5 to 5.5 in the *trans* compartment under the symmetrical conditions had no effect on the channel activity, in particular no change in the current–voltage characteristics of the channel was detected. This result shows that the channel is not permeable for H^+ ions under the conditions of the experiment (10^{-1} M K^+ and 10^{-4}–10^{-6} M H^+).

We found out that the ionic channels induced by fusion of chromatophores with BLM are voltage sensitive, the latter property being more pronounced with fresh chromatophores. Fig. 3 displays an example of the dependence of the open state probability of the channel on the voltage applied. It is seen that the channel became effectively closed down to the state with zero conductance when the voltage exceeded 40 mV irrespective of the sign of the voltage. Fig. 4 illustrates the channel inactivation kinetics calculated by averaging of six current traces.

The voltage inactivation of the channel disappears after the treatment of chromatophores with trypsin. Fig. 5 shows traces of current through BLM before and after 1, 2, 4 min of incubation with trypsin. As shown in [23], the release of the voltage-dependent inactivation by trypsin is a characteristic property of the animal voltage-gated potassium channels.

The ionic channels described here are likely to be rele-

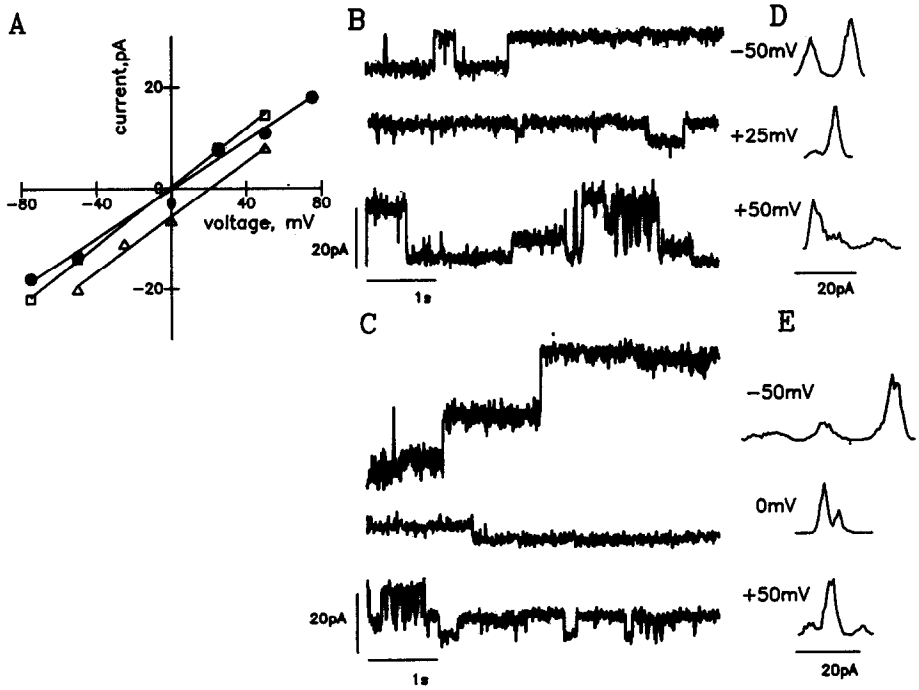

Fig. 2. Current–voltage dependence of the ionic channels in BLM fused with *R. rubrum* chromatophores (A): circles = 100 mM KCl at both sides; triangles = 100 mM KCl at the *cis* side versus 280 mM KCl at the *trans*-side; squares = 100 mM KCl plus 180 mM NaCl at the *cis*-side vs. 280 mM KCl at the *trans*-side. Current traces (B) and amplitude histograms (D) at different values of the voltage applied to BLM under symmetrical conditions (100 mM KCl at both sides). Current traces (C) and amplitude histograms (E) at different values of the voltage applied in the presence of KCl gradient (100 mM KCl at the *cis*-side vs. 280 mM KCl at the *trans*-side of BLM).

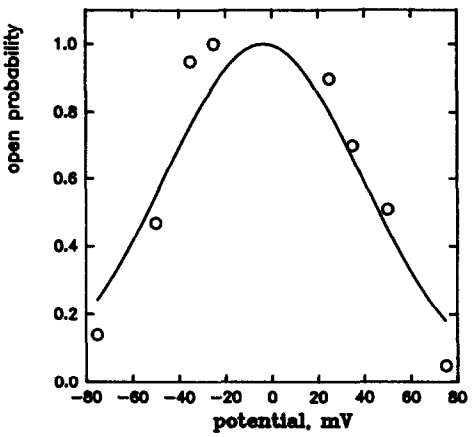

Fig. 3. The dependence of open probability (P_o) of the channel in BLM induced by incorporation of *R. rubrum* chromatophores on the voltage (U) applied under symmetrical conditions (100 mM KCl at both sides).

vant to the process of the establishment of the steady-state electrical potential gradient on the photosynthetic membrane. As it is known, at the onset of illumination of chromatophores an electrical potential difference is generated across the membrane as a result of the operation of the photosynthetic electron transfer chain. The rise in the membrane potential is followed by a partial decrease during illumination, so that at steady state a certain part of energy is stored in the form of pH difference [1]. The mechanism of the membrane potential decline remains obscure. On the basis of estimation of dissipative ionic currents from the initial rate of the decay of the electrochromic absorbance change of carotenoids in *Rhodobacter sphaeroides* cells upon darkening, Golby et al. [24] concluded that the membrane potential decline is due to potential-dependent activation of potassium ion current probably mediated by some transporter similar to TrkA system of *Escherichia coli*. However, in view of our data, one may suggest that the membrane potential decline in the photosynthetic membrane of purple bacteria in the light is promoted by opening of cation-selective ionic channels in chromatophores.

It is worth noting that the existence of large-conductance ionic channels in the photosynthetic membrane is compatible with maintaining of electrical potential difference on this membrane only under the conditions of strict regulation of the channel operation.

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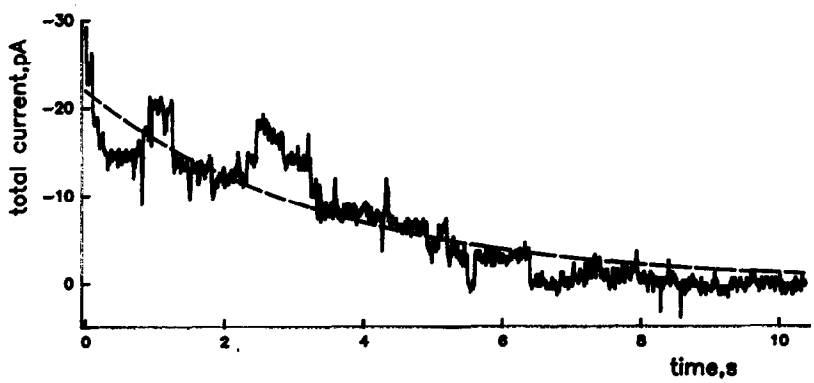

Fig. 4. Kinetics of inactivation of the ionic channels in BLM with *R. rubrum* chromatophores obtained by averaging of 6 current traces (I = a mean current) recorded after application of the voltage of −50 mV under symmetrical conditions (100 mM KCl at both sides). Zero time corresponds to the moment of application of the voltage. The dashed line displays approximation of the averaged experimental curve by the exponential dependence with τ_e = 3.4 s.

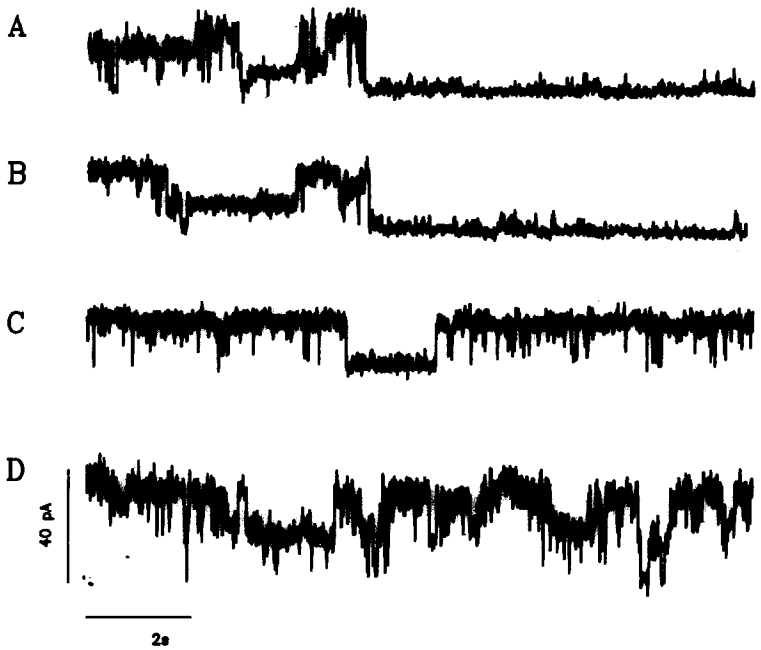

Fig. 5. Traces of current across BLM containing *R. rubrum* chromatophores recorded before (A) and after 1 min (B), 2 min (C) and 4 min (D) of incubation with 1 mg/ml of trypsin added to the bathing solution at both sides of BLM under symmetrical conditions (100 mM KCl at both sides). The beginning of each trace corresponds to the moment of application of the voltage equal to +50 mV.

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